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(54) A MICROBIOLOGICAL METHOD OF PREPARING LIPIDS

(71) We, VSESOJUZNY NAUCHNO-ISSLEDOVATELSKY INSTITUT BIOSINTEZA BELKOVYKH VESCHESTV of 27 Bolshaya Kommunisticheskaya, Moscow, U.S.S.R., a State Enterprise organised and existing under the laws of the U.S.S.R., do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a microbiological method of preparing lipids.

Lipids are fatty substances, which are formed in the metabolic processes in the cells of microorganisms.

Lipids consist of mono-, di- and triglycerides, sterols and their esters, phospholipids, free fatty acids and wax. The lipids may be separated from the cell mass by extraction with an organic solvent, such as benzene, acetone, low boiling point petroleum, low molecular weight hydrocarbons, alcohols, and also chlorine substituted compounds. Lipids may be used for various purposes either individually or in admixture. Thus, lipids may be used for the production of high quality toilet soap or industrial soap, in medical applications and in the food industry. The lipid fatty acids such as oleic and linoleic may be used in paint and varnish production.

A microbiological method for preparing lipids by cultivating microorganisms which produce them, is known. The cultivation is carried out in an aqueous mineral salt and acid medium, which contains as a source of carbon, hydrocarbon petroleum fractions such as paraffin.

One of the components of the medium, nitrogen, is introduced in insufficient quantities or in a form which is difficult for the microorganisms to assimilate. Furthermore, the microorganisms accumulate in their cells up to 30% lipids. A disadvantage of the known method is that it does not produce lipids with the desired structure.

An object of the present invention consists in developing a method of preparing lipids which enables one to regulate the composition of the lipids in accordance with their intended application.

According to the present invention there is provided a method of preparing a lipid comprising aerobically cultivating lipid-producing microorganisms in a nutrient medium, containing mineral salts as sources of nitrogen, phosphorus, potassium and magnesium, and a paraffinic hydrocarbon as a source of carbon, the quantity of paraffinic hydrocarbons not exceeding 75% by volume; maintaining the ratio of nitrogen to carbon no lower than 1:75, and the ratio of phosphorus to carbon, calculating phosphorus as phosphorus pentoxide, no lower than 1:60, and maintaining simultaneously a medium dilution coefficient in the range of 0.5 to 0.07/hr.

Aeration and intensive mixing of the culture medium may be provided in the biosynthesis process, both in the batch system, and in the continuous system.

The composition of the lipids may be changed according to the requirements of its utilization. This change is attained by growing the microorganisms in the technological conditions described below.

Thus, for preparing lipids, which have an enriched phospholipid fraction (up to 50%), and a fraction with enriched free fatty acids (up to 40%) and triglycerides (up to 10%) the biosynthesis process is carried out in a

nutrient medium, in which the content of the carbon source does not exceed 25% by volume; the ratio of nitrogen to carbon, N:C, is in the range 1:5 to 1:20, preferably 1:10; the ratio of phosphorus (calculated as P_2O_5) to carbon, P_2O_5:C, is 1:15 to 1:30, preferably 1:30; with such ratios the dilution coefficient of the medium is kept in the range of 0.1 to 0.07/hr, preferably 0.1/hr. The phospholipid fraction is separated in the known way, and is used chiefly in medicine and the food industry. The free fatty acid fraction may be used in soap production or in the paint and varnish industry.

In order to obtain lipids, which contain as much as 75% by weight of a triglyceride fraction and small quantities of the other components (up to 9% phospholipids, and up to 5% free fatty acids) the growth of the microorganisms is carried out in conditions where the nutrient medium contains not more than 50% by volume of paraffin hydrocarbons; the ratio of nitrogen to carbon in the medium is kept in the range 1:30—45, the ratio of phosphorus (calculated as P_2O_5) to carbon 1:15—30; the value of the medium dilution coefficient being kept in the range 0.1 to 0.07/hr.

Lipids of such a composition may be submitted to fractionation for the separation of their glycerides, which may be used as emulsifiers, particularly in the production of margarine. The glycerides may be saponified for the separation of fatty acids which may be used in soap manufacturing or in the paint and varnish industry.

In order to obtain lipids which contain up to 50% by weight of a free fatty acid fraction, the growth of the microorganisms is carried out in conditions where the nutrient medium contains 50—70% by volume of paraffin hydrocarbons; the ratio of nitrogen to carbon in the medium is in the range 1:5—20; the ratio of phosphorus (calculated as P_2O_5) to carbon is in the range 1:15—30, while maintaining the medium dilution coefficient in the range 0.5 to 0.07/hr. The free fatty acid fraction separated from the lipids may find application, as described above, in soap production and in the paint and varnish industry.

Synthetic or petroleum paraffin hydrocarbons may be used in the above described variants of carrying out the method; also direct petroleum distillation fractions may be used.

The following is a practical way of carrying out the method.

Microorganisms, such as yeast, are grown by the continuous or batch method, in laboratory or industrial conditions, maintaining intensive mixing and aeration of the medium with air.

The microorganisms, such as yeast, are capable of assimilating paraffin hydrocarbons, whether they are present in a mixture with hydrocarbons or another structure, whether they are individual *n*-alkanes or a mixture of them. Therefore, as a source of carbon there may be used in the growth of microorganisms, individual n-alkanes, or mixtures of them; there may also be used synthetic paraffin hydrocarbons and also paraffin hydrocarbon fractions separated from petroleum from different deposits, and distillates of paraffinic and high paraffinic petroleum (for example the diesel oil fraction of petroleum) which contains 20—40% paraffinic hydrocarbons with a straight chain structure.

The aqueous mineral salt and acid nutrient medium must contain ammonium sulfate, phosphoric acid, potassium chloride and magnesium sulfate.

The pH of the medium must be kept in the range of 3—8, the temperature in the range 27°—38°C, and the average expenditure of air 20—200 $m^3/m^3/hr$.

The biological mass is separated from the culture medium and dried at 105°. The lipids are separated from the biological mass by extraction with an organic solvent.

The yield of biological material is 50—75%, with a lipid content of 20—40%. For analytical purposes the lipids were extracted from the biological material using a mixed solvent made up of chloroform-ethanol (1:1). The components of the lipids were determined by thin layer chromatography. The fatty acid content was determined by gas liquid chromatography of their methyl esters using a flame ionization detector.

The research which we carried out allowed us to establish that the nitrogen content in the mineral salt medium affects the composition of the lipids.

The influence of the nitrogen content in the nutrient medium (the ratio of the carbon source to the nitrogen source i.e. C:N, varies from 5:1 to 75:1) on the composition of the lipids was studied in the process of growing the microorganisms. The content of the other components of the mineral salt medium remained constant, in these studies. The dependence of the lipid composition of the yeast on the C:N ratio in the nutrient medium is shown in table No. 1.

From the data given in Table 1, it is obvious that changing the nitrogen concentration in the culture medium, C:N, from 5:1 to 75:1 (while using both purified liquid paraffins and petroleum distillation fractions for growing the microorganisms) the phospholipid content of the lipids decreases, and the biosynthesis of the triglycerides increases. Furthermore, the overall quantity of unsaturated acids, and in particular, linoleic, decreases in the lipids.

It was established that while maintaining the phosphorus source ratio with respect to carbon in the range 1:60 to 1:5, in the nutrient medium, the quantity of lipids remains

the same in the cell mass; however, its composition changes considerably.

The dependence of the lipid composition of the yeast on the ratio, carbon: phosphorus, in the nutrient medium is given in table 2.

TABLE 1

No.	C : N	Lipid content %	Yield yeast % of paraffin	Content main fraction (component) in lipids %		Content, unsaturated acids in the lipids %		
				phospho-lipids	Triglycerides	Overall	Oleic	Linoleic
		Purified liquid paraffins						
1	10:1	14.0	65.0	36.6	17.3	69.9	23.6	11.4
2	15:1	14.2	60.8	32.0	30.0	67.6	16.5	13.3
3	30:1	22.7	58.6	12.0	64.0	66.0	18.9	7.6
4	60:1	37.4	47.7	5.8	64.4	54.4	24.4	3.4
5	75:1	23.9	51.0	10.6	52.6	55.0	16.3	7.5
		Distilled petroleum factions						
6	5:1	8.9	59.0	21.0	41.0	49.7	11.0	5.3
7	10:1	20.7	58.6	12.7	64.0	50.8	9.9	4.7

TABLE 2

$C:P_2O_5$	Lipid content %	Yield organism tissue % of paraffin	Content main fraction components in lipids %		Content, unsturated acids in the lipids %		
			phospho-lipids	Triglycerides	Overall	Oleic	Linoleic
60:1	21.4	55.6	10.7	70.3	54.5	21.4	6.0
30:1	22.7	58.6	12.0	64.0	56.0	18.9	7.6
15:1	22.2	61.9	19.5	54.4	62.5	20.8	10.4

It is seen from the data in Table 2, that, on increasing the content of phosphorus in the nutrient medium (calculated on the basis of P_2O_5), where $C:P_2O_5$ varies from 60:1 to 15:1, the phospholipid fraction in the cell lipids increases simultaneously with the decrease in the proportion of triglycerides.

The overall content of unsaturated acids in the lipids increases. The quantity of linoleic acid in their components also increases to some extent.

Consequently, changing the phosphorus content in the nutrient medium permits the regulation of the quantities of such components in the lipids, as phospholipids, and triglycerides, and also linoleic acid.

On growing microorganisms in nutrient media with different concentrations of paraffin containing petroleum fractions in the range of 10 to 75%, there was noted changes both in the lipid components, and in the fatty acid composition in the lipids.

The fermentation was carried out with $C:N=10:1$, $C:P_2O_5=10:1$, and the medium dilution coefficient=0.1/hr.

The dependence of the composition of the lipids on the concentration of the paraffin fraction is given in table 3.

TABLE 3

Concentration of the distillate fraction in the medium %	Lipid content %	Yield of organism tissue % of paraffin	Content of main component fractions in the lipids %			Content unsaturated acids in the lipids %		
			phospho-lipids	Trigly-cerides	Free fatty acids	Overall	Oleic	Linoleic
10	12.4	55	52.3	19.7	12.5	59.6	7.4	3.5
25	12.0	47	52.5	18.0	7.7	65.2	12.7	9.8
50	11.0	25	5.4	39.2	34.4	65.5	25.6	5.5
75	9.4	12	14.2	22.3	47.8	63.2	9.0	8.2

From the data in table 3 it is seen that it is possible to obtain lipids which contain practically 50% phospholipids if the concentration of the distillate fraction in the medium is in the range 10—25%. Further increase in the concentration of the distillate petroleum fraction in the medium lowers the yield of phospholipids. Regarding the triglycerides a reverse relationship is observed. In the conditions of fermentation which we have chosen, on increasing the concentration of the distillate fraction in the medium up to 50%, there is observed an increase in the quantity of triglycerides from 19.7% to 39.2%, and on further increasing the concentration to 75% there is observed some decrease. Maintaining high concentrations of the distillate fraction in the medium (50—75%), there is observed an increase in the free fatty acid formation.

Consequently, varying the concentration of the distillate fraction in the culture medium permits regulating the composition of the component fractions in the lipids such as triglycerides, phospholipids and free fatty acids. The composition of the free fatty acids may also be regulated: for example, the quantity of oleic acid may be increased to 25.6%.

The dependence of the change in lipid composition on the rate of growth of the organism was studied. This was done by changing the medium dilution coefficient from 0.2/hr to 0.07/hr.

The elucidation, in this way, of the process variables, enabled one to employ definite regimes to regulate the composition of the lipids according to the requirements of the user. Thus, it was shown that changing the medium dilution coefficient, changes the proportion of triglycerides, phospholipids and free fatty acids in the lipids. In the same way there may be changed the proportion of unsaturated acids in the lipids, including oleic and linoleic acids.

In the fermentation conditions we chose, we adjusted the concentration of the petroleum fracion distillate to 10% by vol. and the concentration of purified liquid paraffins to 2% by volume.

The dependence of the lipid composition of the yeast on the magnitude of the medium dilution coefficient is given in table 4.

TABLE 4

No.	D hr.$^{-1}$	Lipid content	Yield organism tissue % of paraffin	Content main components in the lipids %		Content of unsaturated acids in the lipids %		
				phospho-lipids	triglycerides	Overall	Oleic	Linoleic
Purified liquid paraffins (C:N:P_2O_5 = 30:1:1)								
1	0.07	19.8	58.6	12.0	64.0	56.0	18.9	7.6
2	0.1	22.7	58.6	11.4	64.0	67.5	13.3	16.5
3	0.2	28.6	61.0	8.4	66.0	46.5	4.3	14.7
Petroleum fraction distillates (C:N:P_2O_5 = 10:1:1)								
4	0.07	12.0	54.0	33.0	17.7	74.7	26.5	7.2
5	0.1	12.4	43.0	53.0	19.7	56.9	7.4	3.5

From the data given in Table 4 it is seen that on fermentation with purified liquid paraffins, increasing the magnitude of the medium dilution coefficient increases the lipid content in the organism tissue from 19.8% to 28.6%. The yield of the phospholipid fraction, simultaneously decreases somewhat (from 12.0 to 8.6%). The quantity of triglycerides is little dependent on the process rate under the conditions we have chosen for the fermentation.

On using distillation fractions for growing the microorganisms (see table 4), increasing the value of the medium dilution coefficient causes an increase in the phospholipid fraction of the lipids.

The overall content of unsaturated acids, and in particular, of oleic acid, in the lipids of the yeast, decreases with an increase in the medium dilution coefficient.

Consequently, changing the medium dilution coefficient enables one to regulate the composition of the lipids, with regard to their phospholipid content, and also unsaturated acid content, particularly oleic acid.

As seen from the description, the invention enables one to regulate the process of lipid biosynthesis in such a way as to obtain lipids with a predetermined composition.

Thus, it is possible to prepare lipids, which contain up to 50% of a phospholipid fraction. This fraction after being isolated by known methods can be used in the food industry. A particular use in that industry is as an easily assimilable additive in confectionery. It is also used in medicine as a physiologically active component in several medicinal preparations.

Lipids with a high content (up to 75%) of a triglyceride fraction may be used as lubricants in different branches of industry. They also may be substituted successfully for such difficultly available oils as palm oil or coconut oil. By hydrolysis of lipids of such a lipid composition one may obtain fatty acids, which can be used in the paint and varnish industry, as it has been mentioned above. Such lipid fractions can also be used in soap manufacturing for the preparation of high quality toilet and industrial soaps. For this purpose, there can also be used the free fatty acid fraction, which can under certain conditions reach a content of 50% in the lipids.

In the case where petroleum distillates are used for growing the microorganisms, the unassimilated deparaffinated distillate may be used as a winter or arctic motor lubricant.

The organism tissue, after extraction in order to remove the lipids whose content may be as high as 37%, is a valuable animal feed containing up to 60% protein. This protein feed may be used as a protein additive in the food ration of livestock.

The process of growing micororganisms to produce lipids may be carried out batchwise or continuously.

The biosynthesis process is of high productivity since it produces a high yield of organism tissue and it may operate at a high medium flow rate.

The advantage of the invention is that the biosynthesis can be directed to the production of lipids, on an industrial scale.

Examples illustrating the detailed description of the process are given below.

Example 1

Yeast of *Candida tropicalis* is grown in a 30 liter fermenter; the medium, made up of an aqueous solution of mineral salts including those of ammonia, potassium and magnesium, is provided with aeration and intensive mixing. The process is carried out continuously, the degree of medium dilution being from 0.1/hr to 0.07/hr.

The composition of the mineral salt medium is the following: ammonium sulfate 350 mg/l; 70% phosphoric acid 1.2 mg/l; potassium chloride 350 mg/l; magnesium sulfate 175 mg/l. This corresponds to ratios of $C:N:P_2O_5=10:1:1$. The nutrient medium contains 10% by volume of a petroleum fraction, whose paraffins are in the range C_{10}—C_{21}.

The fermentation temperature is ketp at 30—32°C, the pH of the medium at 4—4.5 which is maintained by the additoin of sodium hydroxide solution. The average expenditure of air is 100 m^3/m^3/hour.

The organism tissue is separated from the medium in the presence of small quantities of surface active materials and then dried at 105°C.

The dry yeast is extracted with an organic solvent to remove the lipids. The composition of the latter is given in Table No. 5.

TABLE 5

Medium dilution coefficient per Hr	Quantity of lipids %	Content of main lipid fractions %			Content of unsaturated acids in the lipids %		
		phospho-lipids	Free fatty acids	Triglycerides	Overall	Oleic	Linoleic
0.1	12.4	52.3	12.3	19.7	56.9	7.4	3.5
0.07	12.0	33.0	21.1	17.7	74.7	26.5	7.2

From the data in the table it is seen that changing the medium dilution coefficient from 0.1 to 0.07/hr causes a lowering of the phospholipid content from 52.3% to 33% and a raising of the quantities of free fatty acids from 12.5% to 21.1%. At the same time the quantity of unsaturated acids in the lipids increased from 56.9% to 74.7%, mainly because of an increase in the content of oleic and linoleic acids.

Example 2

Yeast of *Candida pelliculosa* was grown in a mineral salt aqueous medium containing a petroleum distillate fraction. The mineral salt solution contained ammonium, potassium and magnesium salts and phosphoric acid. The fermenter which had a capacity of 30 l, was aerated and provided with intensive mixing.

The degree of medium dilution was 0.1/hr. The concentration of the petroleum distillate fraction was in the range of 10 to 50% by volume; the temperature was held at 30—32°C; the pH was maintained in the range 4—4.5, by the addition of sodium hydroxide solution.

The composition of the mineral salt solution was analogous to that in Example 1, the ratio of carbon to nitrogen in the medium (C:N) was 10:1 and the ratio of carbon to phosphorus ($C:P_2O_5$) was 10:1. The composition of lipids separated from the cell mass by extraction, is shown in Table 6.

TABLE 6

Concentration of distillate fraction %	Quantity of lipids %	Content main fractions in the lipids %			Content of unsaturated acids in the lipids		
		Phospho-lipids	Free fatty acids	Triglycerides	Overall	Oleic acid	Linoleic acid
10	12.4	52.3	12.5	19.7	56.9	7.4	3.5
50	11.0	5.4	34.4	39.2	65.5	25.6	5.5

Increasing the concentration of the paraffin fraction in the nutrient medium from 10 to 50% raises the triglyceride content in the lipids from 19.7% to 39.2% and free fatty acids from 12.5 to 34.4% with a simultaneouse decrease in the quantity of phospholipids from 52.3% to 5.4%.

The quantity of unsaturated fatty acids in the fatty acid fraction increases from 56.9% to 65.5% mainly because of an increase in the quantity of oleic acid from 7.4% to 25.6% and linoleic acid.

Example 3

The yeast *Candida guilliermondii* was grown in a medium of an aqueous mineral salt solution containing a synthetic paraffin mixture (C_{21}—C_{21}). The mineral salt solution contained salts of ammonia, potassium, magnesium and phosphoric acid. The fermenter, whose volume was 17 m³, was provided with aeration and stirring. The process was carried out continuously, with a medium dilution coefficient of 0.1 per hour, a C:P_2O_5 ratio=30:1 and a C:N ratio in the range 15:1 to 45:1.

The composition of the aqueous mineral salt medium was as follows: ammonium sulfate firstly 700 and secondly 233 mg/l; 70% phosphoric acid 0.47 mg/l; potassium chloride 523 mg/l; magnesium sulfate 263 mg/l. The paraffin content in the medium was 1.5% by volume.

The temperature was maintained at 30—32°C, and the pH of the medium at 4—4.5. The average aeration rate was 100 m³/m³/hour. The organism tissue was separated from the nutrient medium, dried at 105°C and the lipids were extracted. The composition of the lipids is given in Table No. 7.

TABLE 7

C:N	Quantity of lipids %	Content of main components of lipids %			Content of unsaturated fatty acids in lipids %		
		Phospho-lipids	Free fatty acids	Trigly-cerides	Overall	Oleic	Linoleic
15:1	14.2	26.2	24.2	32.8	62.4	19.8	10.5
45:1	37.0	8.9	4.2	76.0	35.7	9.4	5.8

From the data in the table it is seen that lowering the nitrogen source content in the nutrient medium from C:N=15.1 to 45:1 (for each part of nitrogen there is not 15, but 45 parts by weight of carbon, i.e. the nitrogen content in the nutrient medium is decreased 3 times) decreases the phospholipid and free fatty acid content in the lipids and increases the triglyceride content from 32.8% to 76%.

The overall quantity of unsaturated acids simultaneously decreases from 62.4 to 35.7%; oleic acid and linoleic acid, in particular decrease as much as 50%.

Example 4

The yeast *Candida tropicalis* is grown in an aqueous mineral salt medium containing purified liquid paraffins (C_{10}—C_{28}) in a 30 l fermenter provided with aeration and stirring.

The ratios of C:P_2O_5 in the nutrient medium were 15:1 and 60:1 in two experiments the C:N ratio being 30:1 in both experiments.

The process was carried out continuously using a medium dilution coefficient of 0.1 hour^{-1}. The composition of the aqueous mineral medium was the following: ammonium sulfate 750 mg/l; 70% phosphoric acid firstly 700 and secondly 180 mg/l, potassium chloride 525 mg/l; magnesium sulfate 263 mg/l. The concentration of paraffin in the medium was 2% by volume, the temperature 30—32°C, the pH 4—4.5, average aeration rate 100 m³/m³/hr.

The organism tissue was separated from the nutrient medium by centrifugation, dried and the lipids extracted with an organic solvent, such as benzine or petroleum ether. The composition of the lipids is given in Table 8.

TABLE 8

$C:P_2O_5$	Quantity of lipids %	Content of the main components in the lipids %			Content of unsaturated acids %		
		Phospho-lipids	Free fatty acids	Trigly-cerides	Overall	Oleic acid	Linoleic acid
15:1	22.2	19.5	8.3	54.4	62.5	20.8	10.4
60:1	21.4	10.7	4.3	70.3	54.5	21.4	6.0

Lowering the content of the phosphorus source in the nutrient medium $C:P_2O_5$ from 15:1 to 60:1 causes a lowering in the phospholipid fraction, and free fatty acids, and increases the triglyceride fraction. The overall unsaturated fatty acids in the lipids decreases, and linoleic acid in particular decreases.

Example 5

Yeast of *Candida tropicalis* was grown on a petroleum distillate fraction with a boiling point 240°—360° in the presence of an aqueous mineral salt and acid solution medium, containing ammonium, potassium, and magnesium and phosphoric acid in a 30 l fermenter provided with aeration and mixing.

The process was carried out continuously with a rate D=0.1/hr, the concentration of the petroleum distillate fraction in the medium being 25% by volume. The ratio C:N=10:1, $C:P_2O_5$=20:1.

The organism tissue was separated from the medium by centrifuging and dried. The lipids were extracted from the dried yeast with an organic solvent. The composition of the lipids according to their main fractions was the following:

phospholipids	52.5%
triglycerides	26.3%
free fatty acids	9.4%

The overall content of unsaturated acids in the lipids was 65.2%, including 12.7% oleic acid and 9.8% linoleic acid.

Example 6

The yeast *Candida guilliermondii* was grown in an aqueous mineral salt and acid solution medium, whose composition was given in Example 3 and which contained n-paraffins, in a 17 m^3 fermenter.

The fermentation was carried out continuously at a rate D=0.07/hr, and a paraffin concentration in the medium of 2% by volume. The ratio C:N=30:1 and $C:P_2O_5$=60.1.

The organism tissue was separated by centrifugation, and dried. The lipids were then extracted with an organic solvent.

The composition of the lipids according to their main fractions were the following:

tryglycerides	70.3%
phospholipids	10.7%
free fatty acids	4.3%

The lipids contained 54.5% unsaturated acids including 21.4% oleic and 6.0% linoleic acids.

WHAT WE CLAIM IS:—

1. A method of preparing a lipid comprising aerobically cultivating lipid-producing microorganisms in a nutrient medium, containing mineral salts as sources of nitrogen, phosphorus, potassium and magnesium, and a paraffinic hydrocarbon as a source of carbon, the quantity of paraffinic hydrocarbons not exceeding 75% by volume; maintaining the ratio of nitrogen to carbon no lower than 1:75, and the ratio of phosphorus to carbon, calculating phosphorus as phosphorus pentoxide, no lower than 1:60, and maintaining simultaneously a medium dilution coefficient in the range of 0.5 to 0.07/hr.

2. A method according to claim 1, wherein a petroleum paraffin fraction is used as the paraffinic hydrocarbon.

3. A method according to claim 1, wherein a synthetic paraffin is used as the paraffinic hydrocarbon.

4. A method according to any one of claims 1 to 3, wherein to prepare lipids which contain up to 50% by weight phospholipids, up to 40% by weight free fatty acids and up to 10% by weight triglycerides, the cultivation process is carried out in a nutrient medium in which the amount of paraffinic hydrocarbon does not exceed 25% by volume, the nitrogen to carbon ratio is from 1:5 to 1:20, the phosphorus to carbon ratio is from 1:15 to 1:30, and the medium dilution coefficient is from 0.1 to 0.07/hr.

5. A method according to any one of claims 1 to 3, wherein to prepare lipids which contain approximately up to 75% by weight triglycerides, up to 9% by weight phospholipids and up to 5% by weight of free fatty acids, the cultivation process is carried out in

a nutrient medium in which the amount of paraffinic hydrocarbon does not exceed 50% by volume, the ratio of nitrogen to carbon is from 1:30 to 1:45, the ratio of phosphorus to carbon is from 1:15 to 1:30, and the dilution coefficient is from 0.1 to 0.07/hr.

6. A method according to any one of claims 1 to 3, wherein in order to prepare lipids which contain up to 50% by weight free fatty acids, and have a low content of phospholipids and triglycerides, the cultivation process is carried out in a nutrient medium, in which the amount of paraffinic hydrocarbon is in the range of 50 to 70% by volume, the ratio of nitrogen to carbon is from 1:5 to 1:20, the ratio of phosphorus to carbon is from 1:15 to 1:30, and the medium dilution coefficient is form 0.5 to 0.07/hr.

7. A method of preparing lipids according to any one of the Examples.

8. Lipids whenever prepared by the method claimed in any one of claims 1 to 7.

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